J. Anat. (1990), **169**, pp. 153–172
With 27 figures
Printed in Great Britain

The cranial cartilages of teleosts and their classification

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(*Accepted* 7 *October* 1989)

INTRODUCTION

The supporting tissues of fish are of special interest to skeletal biologists in general. Studies on acellular and chondroid bone (Moss, 1961; Ørvig, 1951) and on epiphyseal and secondary cartilages (Haines, 1934; Benjamin, 1989*a*) have important implications for those whose primary interest is in mammals. A detailed analysis of skeletal tissues is also needed by ichthyologists concerned with the classification and evolution of fish or with comparative functional morphology. A better understanding of connective tissues could help to develop finer models of explanation (Anker, 1987). Yet, the study of teleostean cartilage has attracted little interest since the work of Schaffer (1930). Few authors are prepared to grapple with the bewildering diversity of tissue types.

In a previous paper I have dealt with the structure and distribution of hyaline-cell cartilage in the heads of teleosts (Benjamin, 1989*b*). In the present paper, attention is focused on the light microscopy appearance of other cartilages, recording the range of tissues for each species, identifying the cartilages that are most characteristic of particular regions of the head and commenting on the interrelationships between cartilage types. In addition, it offers a classification of cartilage that is based on routine, light microscopy sections. A preliminary account of this classification has been presented previously (Benjamin, 1989*c*).

MATERIALS AND METHODS

The serial sections of teleost heads used in the present study (Table 1) are part of an extensive personal collection that is available for viewing by arrangement. One to three animals per species were studied. Only specific names of fish are given in the text, but widely used common names are cited in Table 1. The majority of the fish are small species or young specimens of larger teleosts. They have been bought from commercial suppliers and identified by myself. The standard lengths of the fish are given in Table 1. Many alizarin red/alcian blue (AR/AB) preparations of the skull were made. They greatly aided the naming of bones seen in sectioned material.

The heads were fixed in 10% neutral buffered formal saline, decalcified in 2% nitric acid, dehydrated with graded alcohols, cleared with Inhibisol and embedded in 56 °C Paramat wax or Histowax. Serial transverse, sagittal or coronal sections were cut at 8 μm on a Leitz rotary microtome. Slides were stained with haematoxylin and eosin (H & E), Masson's trichrome, Weigert's and Verhoeff's elastic stains, van Gieson's connective tissue stain, aldehyde fuchsin, and alcian blue and direct red (AB/DR), Line drawings were made with the aid of a Gillert and Sibert projection microscope.

As a pointer to possible developmental relationships between cartilages, a record

was made for each species of gradual transitions that were seen between tissues. The cartilage types could then be ranked according to the frequency with which they merged with other tissues (Table 3).

OBSERVATIONS

Descriptions of cartilages

Hyaline-cell cartilage (HCC) and its subtypes (Figs. 1–4)

HCC is characterised by highly chromophobic, almost translucent cells, that are not shrunken within lacunae (Fig. 1). As the tissue has been described in detail previously (Benjamin, 1989*b*), only a few remarks are needed here for the reader to understand the classification scheme given in Table 4.

Three sub-types of HCC are recognised. Fibrohyaline-cell cartilage (FCC) is a cell-rich fibrocartilage that contains cells typical of HCC, but greater quantities of collagen (Fig. 2). It is not a predominant tissue in any fish. Elastic hyaline-cell cartilage (ECC) is HCC with large quantities of elastic material in the matrix (Fig. 3). It can only be distinguished from HCC by the use of elastic stains. Lipohyaline-cell cartilage (LCC) contains a mixture of hyaline and adipose cells (Fig. 4). It is the rarest of the cell-rich cartilages.

Zellknorpel (ZK) (Figs. 5–7)

The matrix of ZK forms thin seams between adjacent cells and is often more strongly staining than that of HCC. It also gives the impression of being more rigid than the matrix of HCC (it less closely follows the shape of the cells). ZK cells are more chromophilic than those of HCC and are shrunken within lacunae. The ZK in the oral sucker of *Hypostomus* sp. occupies the labyrinthine spaces between the spicules of cancellous bone (Fig. 5). Some of the chondrocytic lacunae in this fish are extremely large (35–40 μm).

Within the gill filaments (rays), ZK is found in the basal plate, its extension (the filament spine plate) and in the filament spine itself (Figs. 6, 7). These terms are defined by Anker (1984). The cartilage of the filament spine plate is often fused with the wall of the afferent branchial artery. It is not merely juxtaposed to the vessel by artefactual shrinkage.

In the filament spine, the chondrocytic lacunae are commonly elongated (Fig. 6). Their long axes are at right angles to the long axis of the filament. The lacunae are very long in *Culaea inconstans* and *Jordanella floridae*, but broader in *Rasbora heteromorpha*. Where adjacent cells are wedge-shaped, the broad bases alternate on

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Fig. 1. Hyaline-cell cartilage in the rostral fold of *Labeo bicolor*. Note the low nuclear:cytoplasmic ratio of the cells and the deeply-indented nuclei (arrows). H & E. × 300.

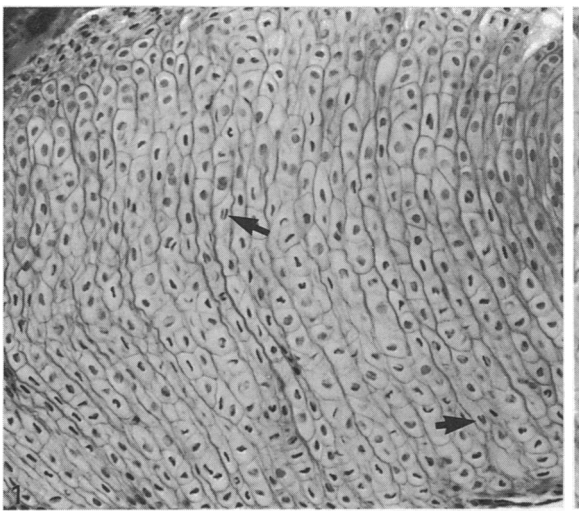
Fig. 2. Fibrohyaline-cell cartilage on the lateral side of the suspensorium in *Botia horae*. It contains prominent bundles of collagen fibres (*C*), interspersed with hyaline cells (*H*). Masson's trichrome. × 500.

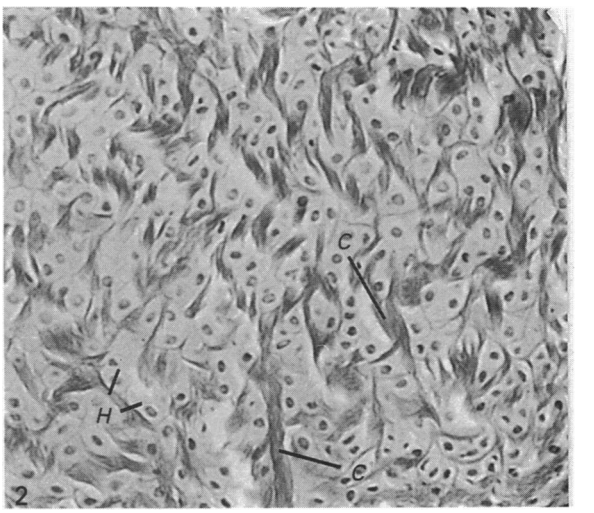
Fig. 3. Elastic hyaline-cell cartilage (*E*) in the oral sucker of *Gyrinocheilus aymonieri*, can easily be distinguished from hyaline-cell cartilage (*H*) in this Weigert's stained section. × 190.

Fig. 4. Lipohyaline-cell cartilage, characterised by the juxtaposition of hyaline (*H*) and fat (*F*) cells, in the oro-mandibular region of *Pseudogastromyzon myersi*. Masson's trichrome. × 190.

Fig. 5. *Zellknorpel* (*Z*) fills the spaces between the spicules of cancellous bone (*C*) in the premaxilla of *Hypostomus* sp. H & E. × 300.

Fig. 6. *Zellknorpel* (*Z*) forming the gill filament spine in *Jordanella floridae*. The cells are greatly shrunken within lacunae and surrounded by perichondral bone (*P*). Masson's trichrome. × 480.

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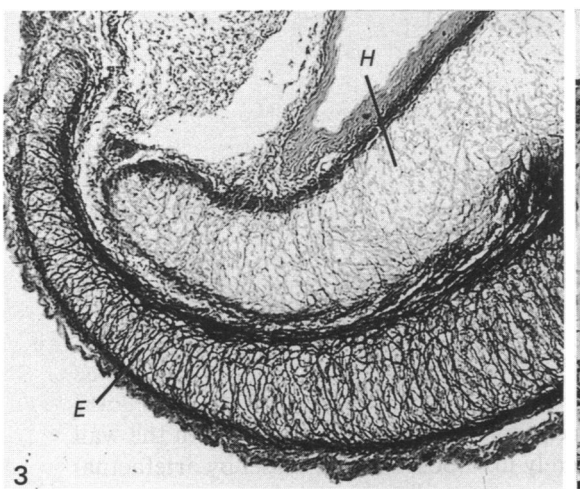
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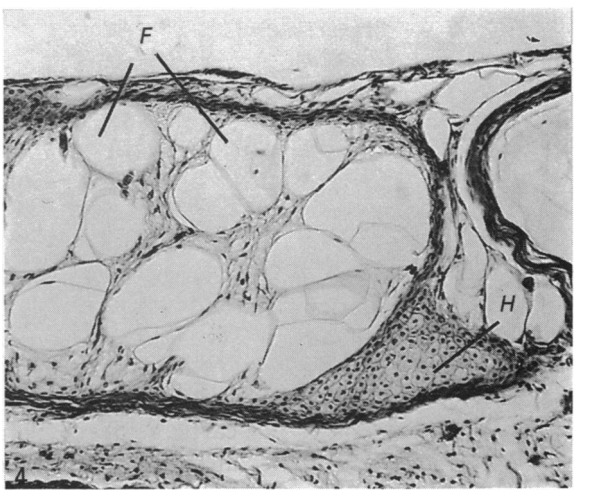
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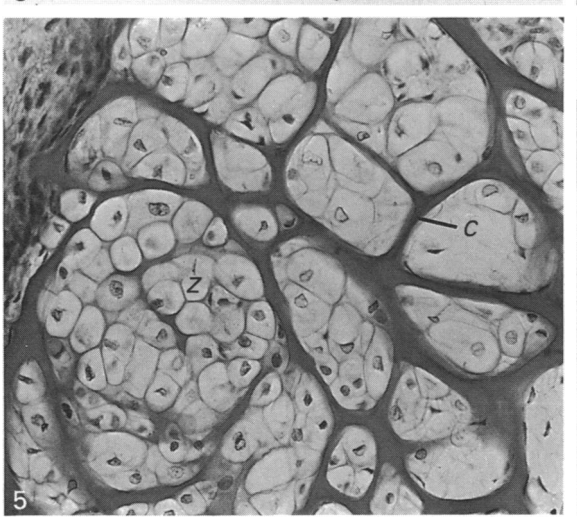
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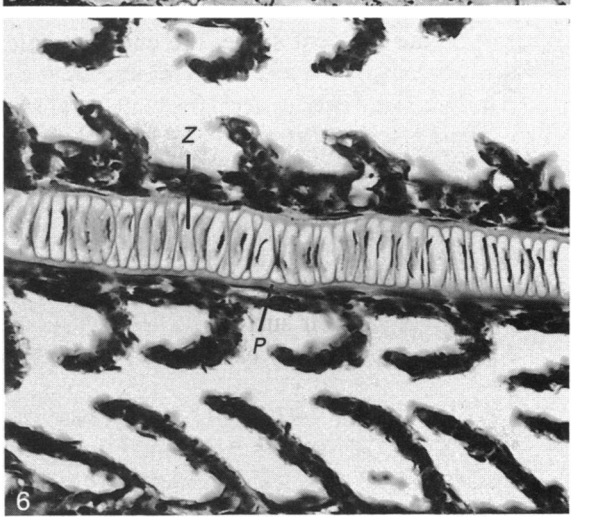
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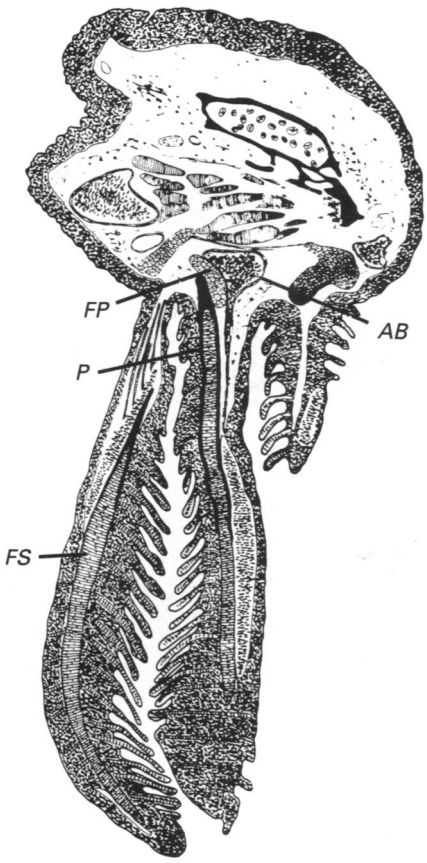

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Fig. 7. A drawing to show the position of the filament spine plate (*FP*) and the filament spine (*FS*) in a gill arch of *Culaea inconstans*. Both the plate and the spine contain *Zellknorpel*. Part of the wall of the afferent branchial artery (*AB*) is replaced by the cartilage of the filament spine plate. Note the perichondral bone (*P*) around the cartilage in the proximal portion of the filament spine.

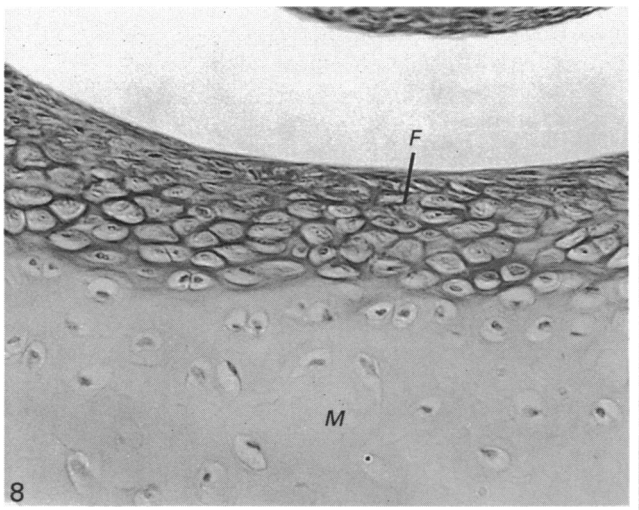
either side of the rod. In *Leuciscus idus*, the cartilage cells have no obvious orientation within the filament rod. They are round/oval and multilayered within each lamella. ZK is also present as a small rod within the pseudobranch of many teleosts, including *Leuciscus idus*, *Rasbora trilineata*, *Geophagus jurupari* and *Telmatherina ladigesi*.

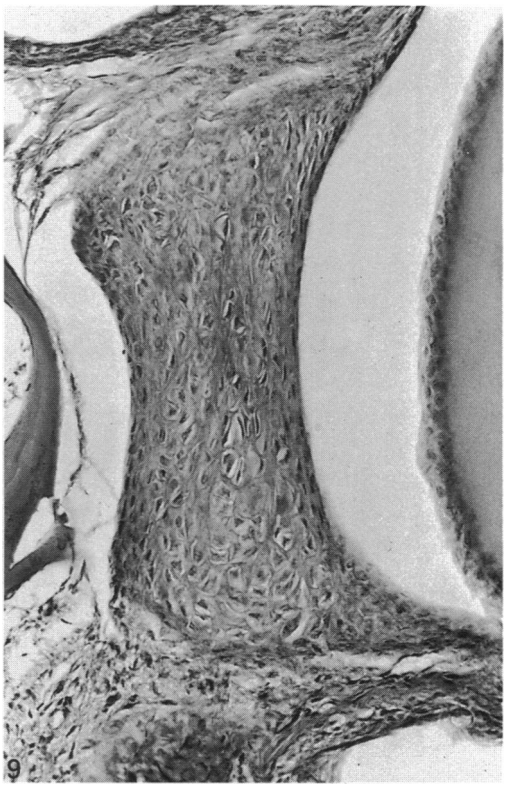
A small amount of ZK (together with some cell-rich hyaline cartilage – see below) supports the opercular valves of *Corydoras paleatus* and *Corydoras aeneus*. It lies on the medial side of the branchiostegal rays, at the attached margin of the valve.

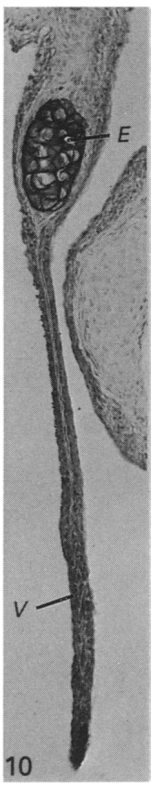
In *Leuciscus idus*, small fragments of ZK are embedded within the dentaries or attached to their surface in the region of the mandibular symphysis. They are either pieces of secondary cartilage or remnants of Meckel's cartilage (see Discussion).

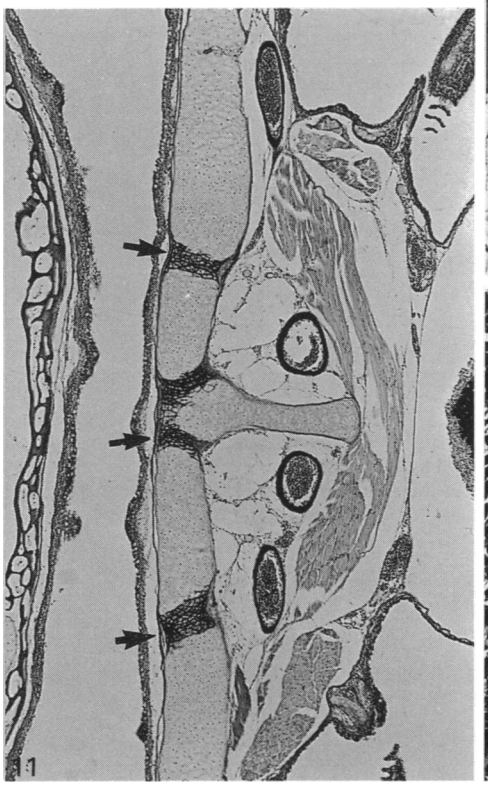
Fibro/cell-rich cartilage (*FCRC*) (*Figs. 8, 9*)

FCRC is a highly cellular fibrocartilage, in which the matrix contains much collagen and the cells are not hyaline. The cells are generally shrunken within lacunae and this helps to distinguish the tissue from dense fibrous connective tissue with which it merges imperceptibly. The cells have a higher nuclear:cytoplasmic ratio than the









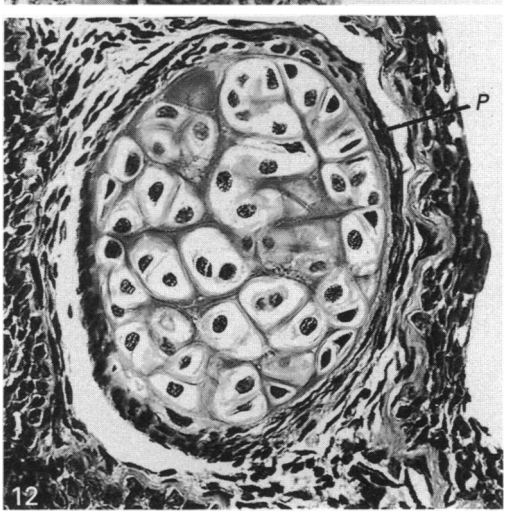


Fig. 8. Fibro/cell-rich cartilage (*F*) and matrix-rich hyaline cartilage (*M*) in the submaxillary meniscus of *Telmatherina ladigesi*. The fibro/cell-rich cartilage is articular. Its numerous collagen fibres make its matrix more darkly staining. Masson's trichrome. ×480.

Fig. 9. Fibro/cell-rich cartilage forming the entire submaxillary meniscus of *Sphaerichthys osphromenoides*. Masson's trichrome. ×300.

Fig. 10. Elastic/cell-rich cartilage (*E*) at the base of the maxillary oral valve (*V*) of *Corydoras paleatus*. Verhoeff's elastic stain. ×120.

Fig. 11. Elastic/cell-rich cartilage (arrows) uniting cartilaginous elements (predominantly CRHC) in the ventral region of the branchiocranium in *Clarias batrachus*. Weigert's elastic stain and van Gieson's connective tissue stain. ×50.

Fig. 12. Elastic/cell-rich cartilage supporting the mandibular barbel of *Pimelodus pictus*. The cartilage is surrounded by a thick perichondrium (*P*). Masson's trichrome. ×480.

cells of FCC. FCRC lacks a discrete perichondrium (i.e. a surrounding envelope of dense fibrous connective tissue, with or without a chondrogenic layer, that is distinct from the cartilage itself) and is never surrounded by perichondral bone.

FCRC is a common articular tissue. It is present, alone or with other tissues, in both the submaxillary meniscus and that between the maxilla and premaxilla of many teleosts, e.g. *Badis badis*, *Macropodus opercularis*, *Telmatherina ladigesi* and *Sphaerichthys osphromenoides* (Figs. 8, 9). In *Culaea inconstans*, FCRC is the articular tissue at the joint between each dorsal hypohyal and the basihyal (Fig. 26). In *Telmatherina ladigesi*, it is found at the articulation of the quadrate with the mandible, and in *Leuciscus idus* and *Corydoras metae*, it is an articular tissue on the hyomandibular.

FCRC forms a symphysial tissue between the two halves of the cleithrum (e.g. in *Macropodus opercularis* and *Jordanella floridae*) and between the hypohyals of *Jordanella floridae*. In *Tetraodon fluviatilis*, the dentaries are largely united at the symphysis menti by a fibrous, yet cellular connective tissue. Although most of the cells are fibroblasts, a few are chondrocytic and closely packed.

FCRC (together with some HCC and loose connective tissue) forms a disc of tissue beneath the keratinised epithelium of the chewing pad in *Rasbora heteromorpha*.

Elastic/cell-rich cartilage (ECRC) (Figs. 10–14)

ECRC is a highly cellular cartilage in which the matrix is rich in elastic fibres and the cells are not hyaline. The cells can be shrunken within lacunae. ECRC can only be distinguished with certainty from ZK or FCRC by the use of elastic stains (Figs. 10, 11). It is frequently surrounded by a thick, fibrous perichondrium (Fig. 12).

ECRC is characteristic of the barbels and maxillary oral valves of catfish. In *Corydoras metae*, it is present in both the bifid mandibular and in the maxillary barbels. In the latter, the cartilages fit into the hollow maxillae. In the oral valves of *Corydoras*, the cartilage is attached to the premaxillae. In *Leiocassis siamensis*, *Clarias batrachus* and *Pimelodus pictus*, the cartilage in the mandibular barbels is continuous with a complex root piece to which fibres of protractor hyoidei are attached (Fig. 13). The root piece is particularly elaborate in *Pimelodus pictus* and *Clarias batrachus*.

In *Clarias batrachus*, part of the wall of the unpaired hypobranchial artery that arises at the distal end of the bulbus arteriosus is formed by ECRC (Fig. 14). The artery is not artefactually pushed against the vessel wall.

Cell-rich cartilage frequently unites the cartilaginous elements in the ventral parts of the splanchnocranium (basi-, hypo- and cerato-(epi-) branchials). Where sections stained for elastic material were available, e.g. *Clarias batrachus* (Fig. 11), it is clear that the joint tissue is ECRC and the branchial elements are hyaline cartilage (both matrix- and cell-rich).

Cell-rich cartilage partly surrounds the olfactory sacs of *Leiocassis siamensis* and *Clarias batrachus*. It extends into the base of the nasal skin flaps in the latter fish. In elastic-stained material (available only for *Clarias batrachus*), the cartilage is identifiable as ECRC.

Cell-rich hyaline cartilage (CRHC) (Figs. 15–17)

CRHC bridges the arbitrary boundary between cell-rich and matrix-rich cartilages in Table 4. It is hyaline cartilage in which $> 50\%$ of the tissue volume is occupied by cells or lacunae (Fig. 15).

Parts of the neurocranium (e.g. in *Botia horae*, *Brachydanio rerio* and *Nannostomus beckfordi*), Meckel's cartilage (e.g. *Macrognathus siamensis*, *Pungitius pungitius* and

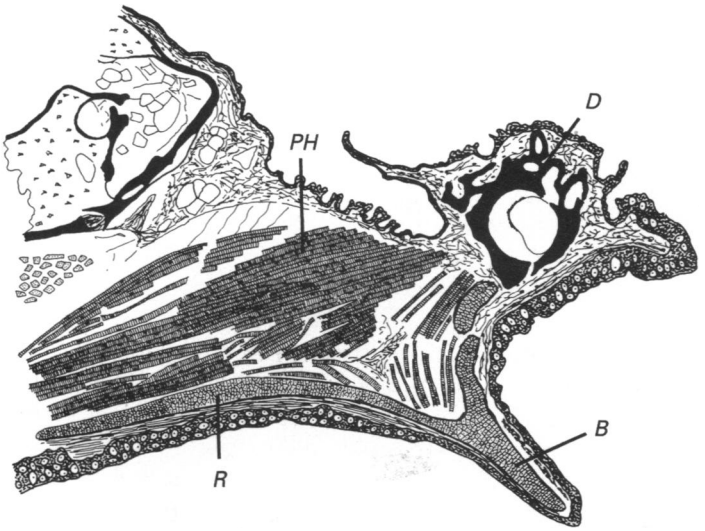


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Fig. 13. A drawing of a sagittal section through the lower jaw of *Leiocassis siamensis* to show the continuity of the elastic/cell-rich cartilage in a mandibular barbel (*B*) with that of the anchoring root piece (*R*). The protractor hyoidei muscle (*PH*) lies deep to the root piece. *D*, Dentary.

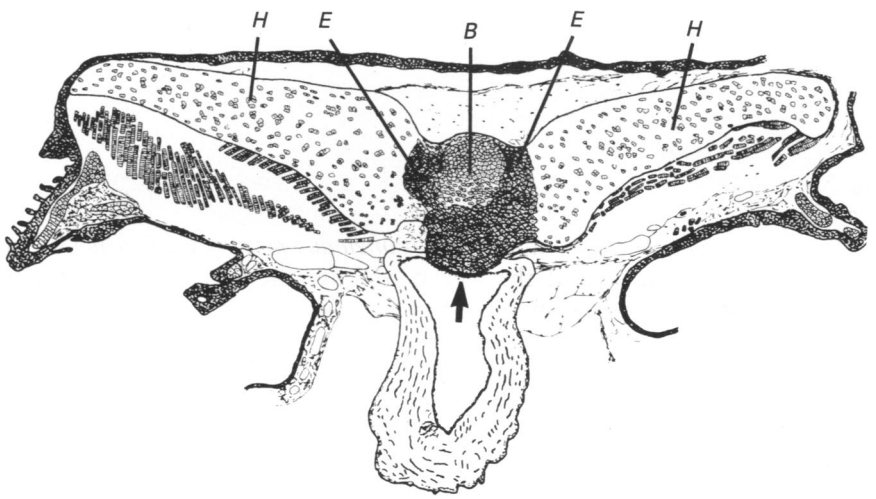

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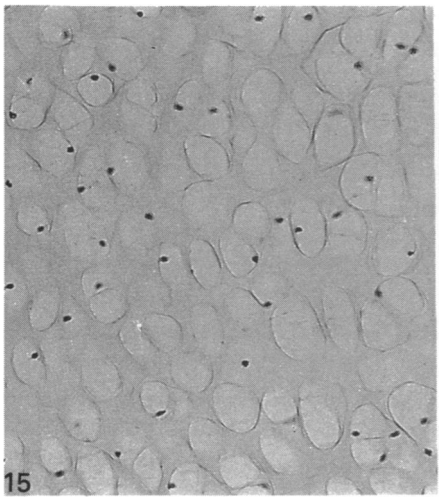
Fig. 14. Elastic/cell-rich cartilage (*E*) in *Clarias batrachus* uniting a basibranchial (*B*) and the paired hypobranchials (*H*) on either side. The same form of cartilage partly replàces the wall of a hypobranchial artery (arrow).

Barbus conchonius) and the branchial elements seen in midline sagittal sections of e.g. *Gyrinocheilus aymonieri*, *Nannostomus beckfordi* and *Epalzeorhynchus kalopterus* are commonly CRHC. There can be great variation in cell size and shape – even in the same piece of cartilage (e.g. in the gill arches of *Nannostomus beckfordi* where the cells range from 5–15 μm in diameter). Some lacunae are particularly large (19 μm) in pharyngobranchial *V* of a 22 mm specimen of *Thayeria boehlkei*. In the occipital region of *Nannostomus beckfordi*, some nuclei in CRHC are sickle-shaped.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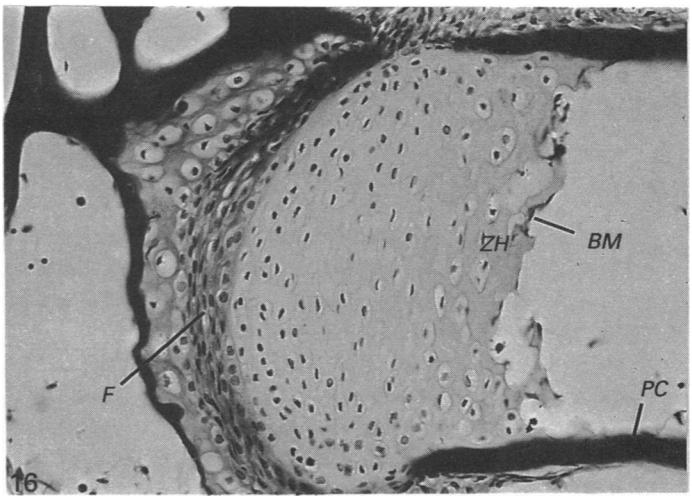

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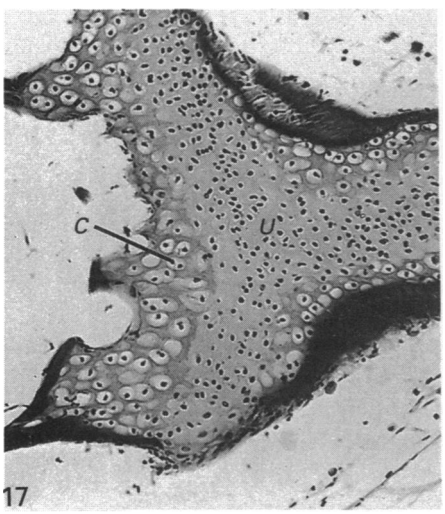

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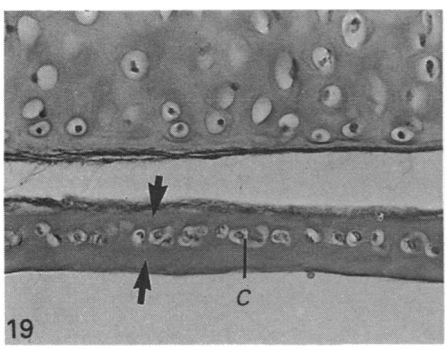

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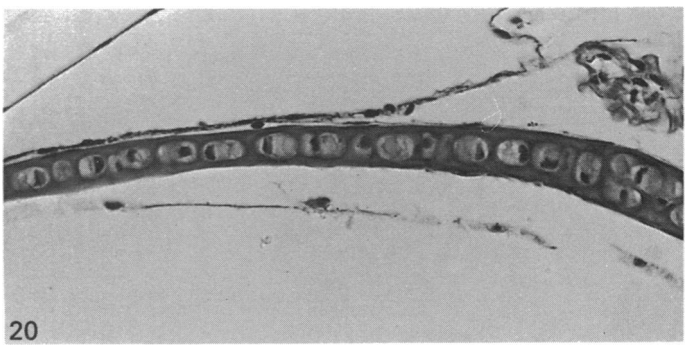
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CRHC frequently forms epiphyseal cartilages. In the hyomandibular epiphysis of *Pimelodus pictus* (Fig. 16), the epiphysial cartilage (CRHC) fits into the bony periosteal tube 'like a cork in a bottle' (Haines, 1934). Many excellent examples of the erosion of chondrocytes during endochondral ossification are provided by CRHC in the splanchnocranium of *Leuciscus idus* and *Pimelodus pictus*. In Figure 17, the

hypertrophic cells are being removed by the destructive action of cells in the adjacent bone marrow. As judged purely by the tinctorial differences in the matrix, calcified CRHC (Fig. 17) is common – particularly in the occipital region.

Matrix-rich hyaline cartilage (MRHC) (Figs. 8, 18, 19)

This is the typical or hyaline cartilage of standard mammalian texts. In routinely-processed material, the chondrocytes are shrunken in lacunae and separated from each other (except in regions of cell nests) by considerable quantities of matrix. The staining properties of the glycosaminoglycans mask those of the collagen fibres. MRHC is common in the neurocranium of deep-headed species (e.g. gouramis, cichlids and trigger fish (Figs. 18, 19)) and is frequently found in gill arches (e.g. *Pungitius pungitius* and *Corydoras metae*). MRHC may be permeated by cartilage canals or channels conveying skeletal muscle fibres. Like CRHC it, too, can be calcified.

Scleral cartilage – a special case (Figs. 19–23)

The sclera can contain or consist of cartilage, dense fibrous connective tissue (DFCT) or bone. Where cartilage is found, it generally forms, or contributes to, a curved plate that surrounds the outer part of the eye. The thickness of the cartilage plate is fairly constant between species (16–19 μm).

Scleral cartilage is difficult to classify, for there is often a central zone of one or two rows of closely-packed cells (making the cartilage CRHC), but these are flanked by peripheral zones of matrix (making the cartilage MRHC). Fish in which a central zone of cells is prominent include *Acanthurus triostegus*, *Pungitius pungitius*, *Cichlasoma nigrofasciatum* (Fig. 19) and *Geophagus jurupari*. In *Tanichthys albonubes*, the scleral cartilage is very cellular throughout, and has no central or peripheral zones (Fig. 20).

Judging from AR/AB preparations, cartilage may rarely be very prominent all around the eye (particularly in certain fish with protruding eyes, e.g. *Pangasius sutchi* (Fig. 21) and *Periophthalmus* sp. – the latter also having prominent extrinsic ocular muscles), but more commonly there are separate pieces of cartilage above and below the eye (e.g. *Botia hymenophysa*, *Gyrinocheilus aymonieri*, *Epalzeorhynchus kalopterus*, *Poecilia reticulata*, *Rasbora trilineata* (Fig. 22) and *Helostoma temmincki*). In *Hyphessobrycon pulchripinnis* (Fig. 23) and *H. herbertaxelrodi*, scleral cartilage is limited to the lower half of the orbit.

Fig. 15. Cell-rich hyaline cartilage in the neurocranium of *Clarias batrachus*. Masson's trichrome. × 300.

Fig. 16. Cell-rich hyaline cartilage forms an epiphysis on the hyomandibular of *Pimelodus pictus* at the articulation of this bone with the skull. Note the zone of hypertrophic cells (*ZH*) and the lack of any visible joint cavity. Fibro/cell-rich cartilage (*F*) is present in the likely plane of movement. *PC*, Perichondral bone collar; *BM*, Thin film of bone marrow. Masson's trichrome. × 300.

Fig. 17. Calcified cell-rich hyaline cartilage (*C*) in a region of endochondral ossification in the otic region of the neurocranium of *Labeo bicolor*. The uncalcified cartilage (*U*) is more lightly staining. Masson's trichrome. × 190.

Fig. 18. Matrix-rich hyaline cartilage in the neurocranium of *Acanthurus triostegus*. *O*, Olfactory sac. Masson's trichrome. × 50.

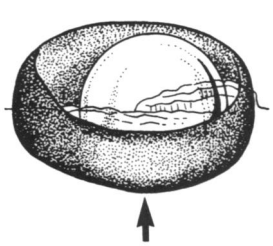
Fig. 19. Scleral cartilage (below) and matrix-rich hyaline cartilage (above) in *Cichlasoma nigrofasciatum*. The scleral cartilage has a central row of closely-packed chondrocytes (*C*) flanked by peripheral zones of matrix (arrows). Masson's trichrome. × 300.

Fig. 20. The scleral cartilage of *Tanichthys albonubes* is more highly cellular than that of *C. nigrofasciatum* (Fig. 19). There are no broad peripheral zones of matrix. Van Gieson's connective tissue stain. × 480.

Table 1. *A summary of the species investigated, their common names, average standard lengths, taxonomic position and cartilage types. Note that elastic-stained material was only available for a few species. A, Hyaline-cell cartilage; B, Fibrohyaline-cell cartilage; C, Elastic hyaline-cell cartilage; D, Lipohyaline-cell cartilage; E, Zellknorpel; F, Fibro/cell-rich cartilage; G, Elastic/cell-rich cartilage; H, Cell-rich hyaline cartilage; I, Matrix-rich hyaline cartilage. ++, predominant; +, present; −, absent.*

Specific name and taxonomic position	Common name and lengths in mm	Cartilage types								
		A	B	C	D	E	F	G	H	I
	Order NOTACANTHIFORMES									
Family MASTACEMBELIDAE										
Macrognathus siamensis	Spiny eel (130)	−	−		−	+	+		++	+
	Order CYPRINIFORMES									
Family CHARACIDAE										
Astyanax jordani	Blind cave fish (27)	+	+		−	+	−		++	+
Hyphessobrycon herbertaxelrodi	Black neon tetra (23)	+	+		−	+	+		++	+
Hyphessobrycon pulchripinnis	Lemon tetra (22)	+	+		−	+	−		++	+
Moenkhausia sanctaefilomenae	Red-eyed tetra (32)	+	−		−	+	+		+	++
Thayeria boehlkei	Penguin fish (22)	−	−		−	+	+		++	+
Family LEBIASINIDAE										
Nannostomus beckfordi	Brown (red) pencil fish (2')	−	−		−	+	−		+	++
Family CYPRINIDAE										
Barbus conchonius	Rosy barb (36)	+	+		+	+	+		++	−
Brachydanio rerio	Zebra danio (39)	+	+		−	+	+		++	−
Brachydanio frankei	Leopard danio (25)	+	+		−	+	+		++	−
Epalzeorhynchus kalopterus	Flying fox (50)	+	+		−	+	+		++	−
Labeo bicolor	Red-tailed black shark (41)	++	+	−	−	+	+	−	++	+
Leuciscus idus	Golden orfe (56)	+	−		+	+	+		++	−
Pseudogastromyzon myersi	Hong Kong Pleco (36)	++	−		+	+	+		+	−
Rasbora heteromorpha	Harlequin fish (24)	+	−		−	+	+		++	+
Rasbora trilineata	Scissor tail (47)	+	−		−	+	+		+	++
Tanichthys albonubes	White cloud mountain minnow (21)	+	−	+	−	+	+	+	++	−
Family GYRINOCHEILIDAE										
Gyrinocheilus aymonieri	Sucking loach (40)	+	+	+	+	+	−		++	+
Family COBITIDAE										
Botia horae	Hora's loach (28)	+	+	−	−	+	−	+	++	−
Botia hymenophysa	Banded loach (54)	+	+		−	+	+		++	+
	Order SILURIFORMES									
Family BAGRIDAE										
Leiocassis siamensis	Bumblebee catfish (45)	+	−		−	+	+		+	+

Family SILURIDAE										
Kryptopterus bicirrhis	Glass catfish (35)	−	−		−	+	+		+	++
Family PANGASIIDAE										
Pangasius sutchi	(41)	−	−	+	−	+	−	+	+	++
Family CLARIIDAE										
Clarias batrachus	(106)	+	+		−	+	+	+	++	−
Family PIMELODIDAE										
Pimelodus pictus	Spotted pimelodus (49)	−	−		−	+	+		+	++
Family CALLICHTHYIDAE										
Corydoras metae	Masked corydoras (32)	+	−	−	−	+	−	+	+	++
Corydoras paleatus	Peppered corydoras (23)	−	−		−	+	+		+	++
Corydoras aeneus	Bronze corydoras (25)	−	−		−	+	+		+	++
Hoplosternum thoracatum	Port Hoplo (45)	−	+		−	+	+		+	++
Family LORICARIIDAE										
Hypostomus sp.	Pleco (49)	+	+		−	+	−		++	+
	Order ATHERINIFORMES									
Family CYPRINODONTIDAE										
Jordanella floridae	American flagfish (26)	+	−		−	+	+		+	++
Family POECILIIDAE										
Poecilia reticulata	Guppy (31)	+	−		−	+	+		+	++
Family ATHERINIDAE										
Telmatherina ladigesi	Celebes rainbow fish (35)	+	+		−	+	+		+	++
	Order GASTEROSTEIFORMES									
Family GASTEROSTEIDAE										
Culaea inconstans	Brook stickleback (44)	+	−		−	+	+		++	+
Pungitius pungitius	Nine-spined stickleback (32)	−	−		−	+	+		+	++
	Order PERCIFORMES									
Family NANDIDAE										
Badis badis	Dwarf chameleon fish (22)	−	+		−	+	+		++	−
Family CICHLIDAE										
Cichlasoma nigrofasciatum	Convict cichlid (23)	−	+		−	+	+		++	+
Geophagus jurupari	Earth eater (40)	+	+		−	+	+		++	+
Family GOBIIDAE										
Periophthalmus sp.	Mudskipper (35)	+	+		−	+	−		+	++
Family ACANTHURIDAE										
Acanthurus triostegus	Surgeon fish (28)	+	+		−	+	+		+	++
Family BELONTIIDAE										
Betta splendens	Siamese fighting fish (42)	+	+		−	+	+		++	+
Macropodus opercularis	Paradise fish (34)	+	+		−	+	+		++	+
Sphaerichthys osphromenoides	Chocolate gourami (37)	+	+		−	+	+		++	+
Family HELOSTOMATIDAE										
Helostoma temmincki	Kissing gourami (32)	+	+		−	+	+		+	++
	Order TETRAODONTIFORMES									
Family TETRAODONTIDAE										
Tetraodon fluviatilis	Green puffer (31)	−	+		−	+	−		+	++



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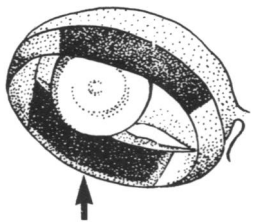

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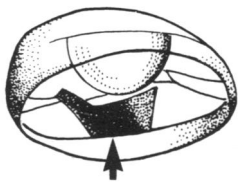

23

Fig. 21. The scleral cartilage of *Pangasius sutchi* as seen in an AR/AB preparation of the skull. The cartilage is prominent and extends all around the eye. The arrow is pointing to the inferior portion of the sclera.

Fig. 22. There are two separate pieces of scleral cartilage above and below the eye in *Rasbora trilineata*. The arrow is pointing to the inferior portion of the sclera. AR/AB preparation of the skull.

Fig. 23. There is a small piece of scleral cartilage below the eye (arrow) in *Hyphessobrycon pulchripinnis*, but none elsewhere. AR/AB preparation of the skull.

In no species was the sclera entirely cartilaginous, for the immediate vicinity of the optic nerve was always dense fibrous connective tissue – presumably to allow the optic nerve to follow the excursions of the eyeball. No cartilage at all was found in the sclera of *Corydoras metae* or *Clarias batrachus*. It is interesting to note that *Astyanax jordani* has underdeveloped eyes and vestigial extrinsic ocular muscle, yet has prominent scleral cartilage.

The range of cartilages in different species

Table 1 summarises the cartilage types seen in the different species and gives the predominant tissue for each fish (usually the cartilage of the neurocranium and gill arches – because of the quantity of tissue present in these locations). The species are arranged in taxonomic order so that the reader can view phylogenetic trends.

CRHC and ZK are the only tissues that were seen in all teleosts. If one disregards elastic cartilages (because only heads from a few species were suitably stained), the rarest tissue was LCC. Among the three orders where the greatest numbers of species were examined (Cypriniformes, Siluriformes and Perciformes), certain trends were particularly evident. HCC and its subtypes were most typical of the Cypriniformes and Perciformes. Within these two orders, FCC best characterised the Perciformes, but HCC itself and LCC were most conspicuous in the Cypriniformes (particularly the cyprinids). Hyaline cartilage (either CRHC or MRHC) was invariably the predominant tissue type in Siluriformes and Perciformes. Although CRHC was found in all Cypriniformes, MRHC was less characteristic of these fish than it was in the other two orders.

The range of cartilages in different regions of the head

Table 2 gives the cartilage type that best characterises different skeletal elements or organs. For each of these elements or organs, one or two species are listed which show the chosen cartilage particularly clearly.

The most cellular cartilages (HCC, ZK and ECRC) are common in places where small pieces of tissue give flexible support (e.g. in barbels, lips and gill filaments). Where a greater bulk of tissue is required, matrix- or cell-rich hyaline cartilage is the rule. Despite the considerable diversity of teleostean cartilage, great conservatism is shown in the type of tissue employed in the neurocranium, gill arches and Meckel's cartilage. In all the teleosts examined, ZK was found in the gill filament spine. Hyaline

Table 2. *Cartilage types that best characterise particular organs or skeletal elements. For abbreviations see text*

Site	Common cartilage type	Examples of species
Barbels	ECRC	*Corydoras metae, Clarias batrachus*
Barbel root piece	ECRC	*Pangasius sutchi, Clarias batrachus*
Rostral appendage	ECRC	*Macrognathus siamensis*
Lips	HCC	*Labeo bicolor, Epalzeorhynchus kalopterus*
Oral valves	ECRC	*Corydoras paleatus*
Opercular valves	CRHC	*Corydoras metae, Corydoras paleatus*
Gill arches	CRHC	*Botia horae, Helostoma temmincki*
Gill filaments	ZK	*Cichlasoma nigrofasciatum, Pungitius pungitius*
Meckel's cartilage	CRHC	*Geophagus jurupari, Tanichthys albonubes*
Neurocranium	CRHC & MRHC	*Thayeria boehlkei, Macropodus opercularis*
Chewing pad	FRCS	*Rasbora heteromorpha*
Olfactory sacs	ECRC	*Clarias batrachus*
Menisci	FRCR	*Macropodus opercularis, Sphaerichthys osphromenoides*
Articular cartilage	FCRC	*Culaea inconstans, Telmatherina ladigesi*
	HCC	*Labeo bicolor, Pseudogastromyzon myersi*

cartilage (CRHC or MRHC) was found in the gill arches themselves, in the neurocranium and in Meckel's cartilage.

Interrelationships of tissues (Figs. 24–27)

Table 3 summarises the frequency with which one tissue merges imperceptibly with another. The most 'labile' tissue is HCC and the most 'stable' is MRHC. All cartilages form gradual transitions with DFCT. Chondroid bone (referred to in Table 3) is regarded as a transitional tissue with properties intermediate between those of cartilage and bone. Although the present study has not dealt with sequential developmental stages of fish, it seems likely, from the gradual merging of one tissue with another, that chondroid bone can be formed from the more highly cellular cartilages by chondroidal osteogenesis (Fig. 24).

Examples of cartilages that are continuous with each other include HCC, ZK and CRHC in the gill arches of *Leiocassis siamensis* (where the branchial elements articulate with the skull (Fig. 25)), and CRHC and MRCH in the basibranchials of *Culaea inconstans*. The periphery of articular cartilage blends with ligamentous fibres at the joint between the basihyal and the dorsal hypohyals of *Culaea inconstans* (Fig. 26). CRHC, ZK and fibroblastic mucous connective tissue (fibroblastic muco-chondroid) merge together in the opercular valve of *Corydoras* sp. (Fig. 27).

A new classification scheme

Based on the descriptions given above, a new classification of teleostean cartilage is proposed in Table 4. It seeks to provide a reference point for future expansions and modifications. Excluded from the scheme are the chordoid and mucochondroid tissues of Schaffer (1930) and chondroid bone.

DISCUSSION

Schaffer (1930) laid great emphasis on the shrinkage of cartilage cells within lacunae as a means of distinguishing 'true' cartilage from cartilage-like or chondroid tissues. However, studies on the hypertrophic cells of epiphyseal growth plates have suggested

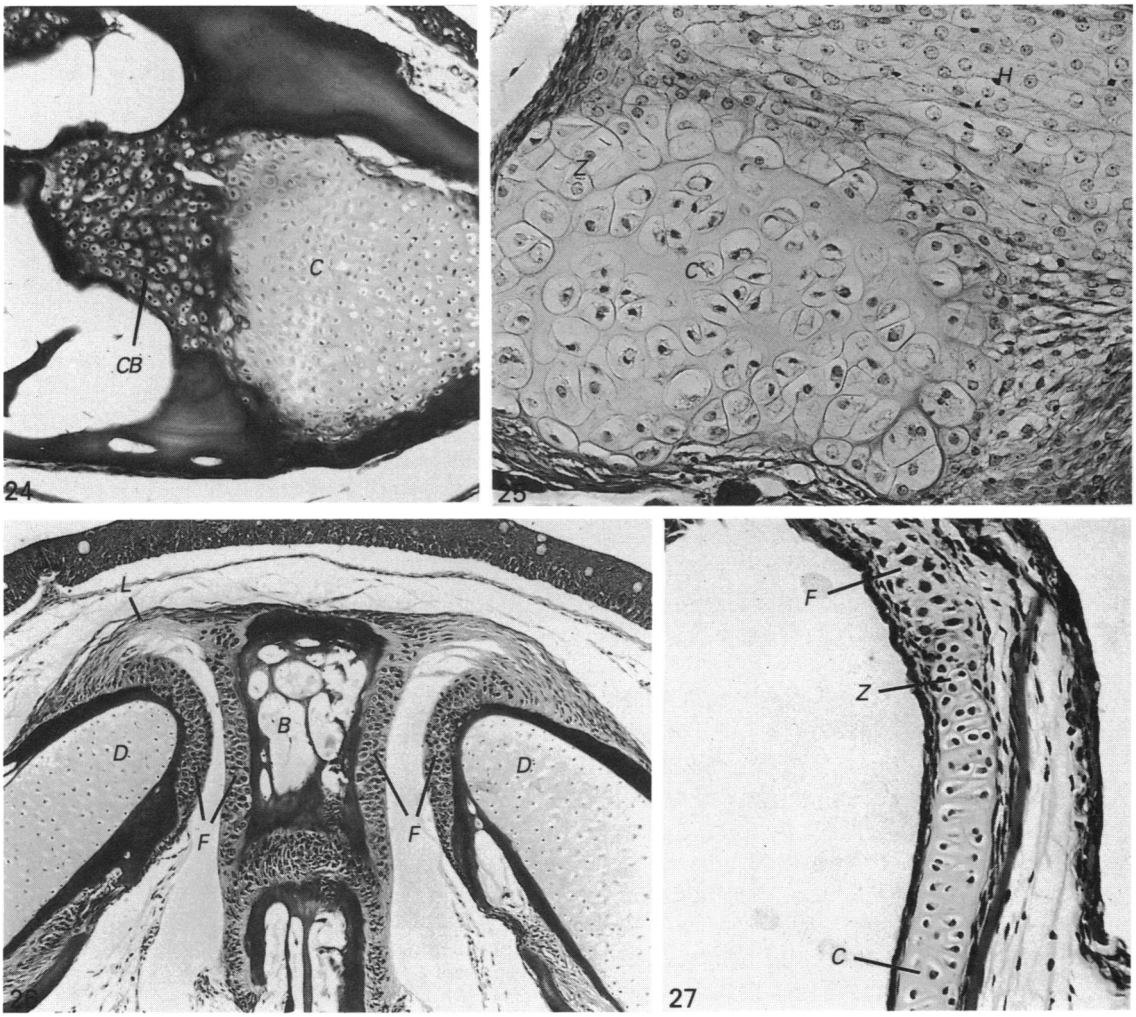

Fig. 24. Chondroidal osteogenesis. Cell-rich hyaline cartilage (*C*) is forming Type II chondroid bone (*CB*) in the anguloarticular of *Sphaerichthys osphromenoides*. Masson's trichrome. × 190.

Fig. 25. A gradual transition between cell-rich hyaline cartilage (*C*), *Zellknorpel* (*Z*) and hyaline-cell cartilage (*H*) in a pharyngobranchial of *Leiocassis siamensis*. Masson's trichrome. × 300.

Fig. 26. A gradual transition between the dense fibrous connective tissue of the ligamentum interhypohyale (*L*) and the articular fibro/cell-rich cartilage (*F*) on the dorsal hypohyals (*D*) and the basihyal (*B*) of *Culaea inconstans*. Masson's trichrome. × 120.

Fig. 27. A gradual transition between fibroblastic mucous connective tissue (*F*), *Zellknorpel* (*Z*) and cell-rich hyaline cartilage (*C*) in the opercular valve of *Corydoras paleatus*. Masson's trichrome. × 190.

that such shrinkage is artefactual. Careful processing can prevent it (Hunziker, Herrmann & Schenk, 1983; Eggli, Herrmann, Hunziker & Schenk, 1985; Akisaka, Subita, Kawaguchi & Shigenaga, 1987). Chondrocytes shrink when they become detached from their pericellular matrix. Akisaka *et al.* (1987) suggest that detachment occurs when the proteoglycans of the pericellular matrix are extracted and the cytoskeleton destroyed. The view that chondrocytic shrinkage is artefactual supports the contention of Person & Philpott (1969) that Schaffer's (1930) concept of true cartilage cannot be maintained.

Table 3. *Imperceptible tissue boundaries between cartilages and between each of them and other tissues. The cartilage types are ranked* 1–6 *according to the frequency with which imperceptible boundaries were seen. For each of these* 6 *cartilage types, the most common boundaries are specified in decreasing order* 1–4. *The two forms of elastic cartilage are excluded from this comparison because of the limited number of fish for which elastic stained material was available. CB, Chondroid bone; DFCT, Dense fibrous connective tissue*

Rank	Cartilages	Commonest boundaries (ranked 1–4)
1	Hyaline-cell cartilage (HCC)	1 = DFCT, 2 = CRHC, 3 = FCC, 4 = CB, 4 = ZK
2	Cell-rich hyaline cartilage (CRHC)	1 = DFCT, 2 = HCC, 2 = ZK, 4 = FCRC
3	Fibro/cell-rich cartilage (FCRC)	1 = CRHC, 1 = CB, 1 = DFCT, 4 = MRHC
4	*Zellknorpel* (ZK)	1 = CRHC, 2 = DFCT, 3 = HCC, 4 = CB
5	Fibrohyaline-cell cartilage	1 = DFCT, 2 = HCC, 3 = CRHC, 4 = CB
6	Matrix-rich hyaline cartilage (MRHC)	1 = CRHC, 1 = DFCT, 3 = FCRC, 4 = HCC

Table 4. *A classification of teleostean cartilage that aims to provide a reference point for future expansions or modifications. Excluded from the scheme are the chordoid and mucochondroid tissues of Schaffer* (1930) *and chondroid bone.* * *Cartilage included for completeness, but not seen by the author in small/young teleosts*

(A) Cell-rich cartilage (cells or lacunae occupy > 50% of tissue volume)	
1. Hyaline-cell cartilage	The *hyalinzellige Chondroidgewebe* of Schaffer (1930). A highly-cellular cartilage where the cells have abundant, chromophobic 'hyaline' cytoplasm. Example = Lips and labial folds of bottom-dwelling cyprinids. Subtypes include: (*a*) *Fibrohyaline-cell* (Fibrocartilage with hyaline cells). Example = Lateral to suspensorium of *Botia horae*. (*b*) *Elastic hyaline-cell* (Elastic cartilage with hyaline cells). Example = Parts of oral sucker of *Gyrinocheilus aymonieri*. (*c*) *Lipohyaline-cell* (A tissue where hyaline and fat cells are intermingled). Example = Labial folds of *Pseudogastromyzon myersi*.
2. *Zellknorpel*	Corresponds to the tissue described by Schaffer (1930). The closely-packed cells are separated from each other by thin bars of matrix. Cells shrunken in lacunae in routinely-processed material. Example = Gill filaments of most teleosts.
3. Fibro/cell-rich cartilage	Highly-cellular fibrocartilage where the cells are not hyaline. The abundant collagen fibres are not masked by the staining properties of the matrix. Example = Intra-articular menisci of numerous teleosts.
4. Elastic/cell-rich cartilage	Highly-cellular elastic cartilage where the cells are not hyaline. Can only be distinguished from *Zellknorpel* in sections stained for elastic material. Example = Barbels of numerous catfish.
5. Cell-rich hyaline cartilage	Resembling hyaline cartilage, but more cellular. Bridges the artificial distinction between matrix and cell-rich cartilages. Example = Neurocranium and gill arches of many teleosts.
(B) Matrix-rich cartilage (cells or lacunae occupy < 50% of tissue volume)	
1. Matrix-rich hyaline cartilage	'Typical cartilage' – the best approximate to a form of human cartilage. Example = Neurocranium of many teleosts.
2. * Fibrocartilage	Most fibrocartilages in small/young teleosts are cell-rich.
3. * Elastic cartilage	Most elastic cartilages in small/young teleosts are cell-rich.

Yet there is no reason why a reliable shrinkage artefact in paraffin-embedded material cannot be used to distinguish different forms of cartilage. Microscopists have long recognised tissues by artefactual appearances – the extraction of lipid from fat cells produces a characteristic pattern that is useful in identifying adipose tissue. The comparative histologist still needs to distinguish between Schaffer's (1930) 'true' cellular cartilage (*Zellknorpel*) and his hyaline cell chondroid tissue (*hyalinzellige Chondroidgewebe*). The cells consistently shrink in the former tissue, but not in the latter. It is too simple to dismiss the distinction as artefactual. Why is only one tissue affected in this way by routine processing? There must be critical differences in matrix composition and/or cell structure.

I have recently proposed that we rename Schaffer's (1930) *hyalinzellige Chondroidgewebe* 'hyaline-cell cartilage' (Benjamin, 1989*a*). There is no reason to deny its cartilaginous status. While, for example, there are sufficiently pronounced differences in the matrix of the elastic/cell-rich cartilage in *Macrognathus siamensis* (Benjamin & Sandhu, 1990) and hyaline-cell cartilage in *Gyrinocheilus aymonieri* (Benjamin, 1986) to warrant their separate identification, the overriding ultrastructural similarity in their cells suggests that there is no basis for continuing to regard the former as 'true' cartilage and the latter as 'chondroid' tissue.

The term *Zellknorpel* (literally 'cellular cartilage') unfortunately implies that there are acellular cartilages. Although acellular zones are described here in the sclera, I have seen no cartilages that are completely devoid of cells. However, *Zellknorpel* is retained in the present classification, with the meaning that Schaffer (1930) attached to it, in order to preserve an essential continuity between Schaffer's (1930) scheme and mine. The reader should note however, that '*Zellknorpel*' is not synonymous with the term 'cell-rich cartilage' used in the present paper. In Schaffer's (1930) *Zellknorpel*, the cells are encapsulated by thin seams of matrix (hence the alternative name – *cartilage à stroma capsulaire*). My 'cell-rich cartilage' is a far broader category that includes *Zellknorpel* and other cartilages in which the cells or their lacunae occupy more than 50% of the tissue volume. I feel that the similarities between cartilages with a small amount of inter-territorial matrix and those that can strictly be called *Zellknorpel* greatly outweigh their differences. Schaffer's (1930) scheme leaves one with numerous highly-cellular cartilages which must still be called 'matrix-rich'. They are called cell-rich hyaline cartilage here.

The organisation of *Zellknorpel* chondrocytes in the filament spine of most teleosts is very orderly. The cells are arranged like coins in a pile, with each cell neatly stacked on top of the next. The arrangement recalls that of the proliferation zone in an epiphyseal plate of a developing mammalian long bone. It is likely too, that the arrangement of the cells in the filament spine provides for growth in length of the gill filament – along the long axis of the filament spine.

The presence (but not the histological type) of cartilage in barbels is well known (Hoffmann, 1923; Kapoor & Bhargava, 1967; Singh & Kapoor, 1967; Saxena & Aggarwal, 1971; Joyce & Chapman, 1978) and has been used as a basis for their classification. Sato (1937) recognised catfish-, goatfish- and loach-type barbels with cartilage and carp-type barbels without cartilage. Although Sato's study and that of Benjamin (1989*b*), show that some loaches have cartilage in their barbels (e.g. *Misgurnus anguillicaudatus* and *Heteropneustes fossilis*), other loaches do not (e.g. *Botia horae* and *Botia macracantha*). Indeed Sato & Kapoor (1957) later reported the absence of cartilage in *Botia lohachata*. The present finding of elastic cartilage in catfish barbels, contrasts with a previous report of Ghiot & Bouchez (1980). They were puzzled by the supporting tissue in the barbels of *Pimelodelus clarias* and *Ictalurus*

nebulosus. They showed that the rod reacted with elastic stains, but could not see any chondrocytes. I suspect this is because they only used an elastic stain to study the barbels.

The rootpiece that anchors the barbels in *Leiocassis siamensis* and *Pimelodus pictus* to the protractor hyoidei muscle is most intriguing. It provides a basis for the active movement of barbels that lack skeletal muscle fibres within them. The plate was considered in detail in a wide range of animals by Pollard (1894). He saw striking parallels between the whole system of root pieces, barbels, nerves and muscles in *Myxine* and various teleosts (especially siluroids), with the oral cirri of *Amphioxus*. The root piece was regarded by Norman (1926) as part of the premandibular complex in siluroid fishes (see below). A similar plate of tissue has been described by Hoffmann (1923) in *Siluris glanis* anchoring the barbels to geniohyoid (= protractor hyoidei according to Winterbottom (1974)). Hoffmann (1923) speculates that the cartilage arises either by fascial transformation, or from Meckel's cartilage. The proximal end of the rod in the maxillary barbels of *Pimelodus pictus* is anchored in a hollow of the bone, as it is in *Pimelodelus clarias* (Ghiot & Bouchez, 1980).

In the anterior region of the head of many bony fish, there are certain paired or unpaired cartilages that perhaps correspond to the labial cartilages of Selachians (Norman, 1926). As these in turn are thought to represent the remains of the premandibular visceral arches, the teleostean cartilages are sometimes called premandibular elements. They include the menisci associated with the premaxillae and maxillae. According to Norman (1926), the premandibular complex also includes the paired labial cartilages that are found in the angles of the mouth in certain teleosts, and the premaxillary cartilages that may fuse to form a median rostral cartilage. These elements are HCC in many teleosts, particularly cyprinids (Benjamin, 1989*b*). However, other cell-rich cartilages are here reported in the menisci of *Badis badis*, *Macropodus opercularis*, *Telmatherina ladigesi* and *Sphaerichthys osphromenoides*.

Norman, (1926) has commented on the comparatively late development of the premaxillary cartilage in *Salmo* – after the first appearance of the premaxillae. They are thus secondary cartilage – a tissue that until recently was regarded as restricted to higher vertebrates (Benjamin, 1989*a*). Nigrelli & Gordon (1946) have reported an osteochondroma in the jewel cichlid, *Hemichromis bimaculatus*. The tumour affected the maxilla and a number of opercular bones. They describe the tissue as hyaline cartilage. However, in both their illustrations and descriptions, they refer to highly cellular regions of the tumour. Again, this is secondary cartilage. Although the authors did not recognise it as such, they did comment on the chondrogenic properties of the periosteum.

Scleral cartilages are widespread in the animal kingdom. Schaffer (1930, p. 345) refers to them in cephalopods, urodeles, anurans, sauropsidans, monotremes, selachians, salamanders, lampreys, teleosts. lizards and amphibians among others. More recently, the cartilages have been reviewed by Hall (1978) and it is clear that little attention has been directed to the teleostean sclera.

Schaffer (1930) discussed scleral cartilages under the heading *Das grundsubstanz-reiche Knorpelgewebe mit verästelten oder spindeligen Zellen* (pp. 242–257). This unwieldy phrase translates as 'ground substance-rich cartilage tissue with branching or spindle-shaped cells'. It is a tissue that was regarded by Schaffer (1930) and his eminent predecessors (Langer, Gegenbaur, Koelliker and Muller), as distinct from hyaline cartilage. Nevertheless, Schaffer's (1930) Figures 210 and 211 clearly show that the scleral cartilage of *Sepia* is packed with cells. To Schaffer (1930), the similarity in the staining properties between scleral and matrix-rich cartilages outweighed any

differences in cellularity. The scleral cartilages of teleosts attracted Schaffer's (1930) attention because of their branching cells. However, this feature is not often striking, even in sticklebacks, one of the fish he mentioned (p. 245). Branching cells are most easily seen in grazing sections through the cartilage. The cells must therefore be forming a stellate network in the plane of the curved sheet of cartilage.

In the teleosts I examined, there is frequently a prominent matrix-rich layer near the external and internal surfaces of the scleral cartilage, and a scanty perichondrium. Such acellular zones are particularly striking in *Acanthurus triostegus*. According to Schaffer (1930, p. 248) the sclera of *Lophius* (angler fish), *Ramphistoma* and *Rana mugiens* have similar zones.

Cartilage is occasionally reported in teleosts in the region where the dentaries meet in the midline, though its histological type is not discussed. Howes & Sanford (1987) have described symphysial and post-symphysial cartilages in the lower jaw of *Plecoglossus altivelis* and made the interesting observation that such cartilages contribute to the formation of symphysial processes on the dentary. Cartilage is also present on the dentaries, near the symphysis menti, in adult *Oryzias latipes* (Langille & Hall, 1987). Their comprehensive developmental study shows it to be a remnant of Meckel's cartilage. It is possible that the small pieces of cell-rich cartilage found in the symphysial region of *Leuciscus idus* are also persistent portions of Meckel's cartilage that have separated from the main bulk of the rod located more posteriorly. The alternative view is that it is secondary cartilage. Such a tissue has been described in the symphysis menti of the hamster (Trevisan & Scapino, 1976).

The inter-relationships of teleostean cartilage are exceedingly complex. The frequency with which one tissue merges imperceptibly with another is taken as the best available indication of the developmental relationships between the different cartilage types and between them and related tissues. However, one cannot state directly which cartilage develops into which, without studying developmental series. Yet, it seems probable that cell-rich cartilages develop into matrix-rich ones and not *vice versa* and that matrix-rich hyaline cartilage is the most stable phenotype. It is also likely that HCC and ZK do not differentiate from the two fibrocartilages (FCC and FCRC), but that they may develop into them (Benjamin, 1989*b*). Imperceptible boundaries with DFCT are probably common because DFCT connects many cartilages to adjacent structures. HCC is a fully differentiated tissue in some animals (Benjamin, 1989*b*), but a stage in the development of further cartilages in others. It is likely to be the most fruitful tissue for further investigations of the developmental and phylogenetic relationships of cartilage in lower vertebrates. It is a key tissue in considering the diversity of teleostean cartilage and the conservatism of mammalian tissue.

The power and precision of Schaffer's (1930) classification of cartilage and related tissues is unrivalled, but his scheme is in German and it compares a wide range of vertebrate and invertebrate tissues side by side. The present classification is restricted to teleosts and seeks to provide a foundation for future expansions or modifications. The reader should note that the classification deals with the histological appearance of the tissues in routine, light microscopy sections. Thus, it does not include precartilage or secondary cartilage. These terms are valid and useful, but do not refer primarily to morphology. Neither does it include mucocartilage. As discussed elsewhere (Benjamin, 1988), I favour the terms 'mucous connective' or 'mucochondroid', for the gelatinous tissue that some authors call mucocartilage. Difficulties with my classification may arise if it is applied to resin-embedded material (Anker – personal communication), for the staining properties of the tissues may differ and lacunar shrinkage may be less apparent.

SUMMARY

The structure and distribution of cartilages has been studied in 45 species from 24 families. The resulting data have been used as a basis for establishing a new classification. A cartilage is regarded as 'cell-rich' if its cells or their lacunae occupy more than half of the tissue volume. Five classes of cell-rich cartilage are recognised (*a*) hyaline-cell cartilage (common in the lips of bottom-dwelling cyprinids) and its subtypes fibro/hyaline-cell cartilage, elastic/hyaline-cell cartilage and lipo/hyaline-cell cartilage, (*b*) Schaffer's *Zellknorpel*, typified by the cartilage in the gill filaments of most teleosts examined, (*c*) elastic/cell-rich cartilage, such as that which supports the barbels and oral valves of catfish, e.g. *Corydoras metae*, (*d*) fibro/cell-rich cartilage, as in the submaxillary meniscus of *Sphaerichthys osphromenoides*, (*e*) cell-rich hyaline and (*f*) matrix-rich hyaline cartilage – both of which are common in the neurocranium and gill arches of most teleosts.

The range of cartilages seen, and the predominant cartilage type, is recorded for each species and a list is provided of the tissues that most typify different organs or regions of the head. As a preliminary pointer to developmental relationships between the cartilages, note was taken of gradual transitions between one cartilage and another. It is suggested that hyaline-cell cartilage occupies a key position in teleosts as the most labile of the supporting tissues and is highly characteristic of Cypriniformes. The cartilage that best resembles mammalian hyaline cartilage (matrix-rich hyaline cartilage) has a very conservative distribution in different skeletal elements and the least number of associations with other tissues. It is well represented in Siluriformes.

I wish to thank Dr G. Ch. Anker for his most helpful comments on an early version of the manuscript and Mr D. Scarborough for cutting the light microscopy sections.

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